

10T1/2 Cells: An *In Vitro* Model for Molecular Genetic Analysis of Mesodermal Determination and Differentiation

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Progress has been made in understanding the molecular mechanisms that regulate cell type-specific gene expression during the terminal differentiation of cells into specialized tissue types. These studies have concentrated largely on defining the *cis* elements and *trans*-acting factors responsible for the transcription of differentiation-specific genes. Valuable as these investigations have been, they have not been able to place differentiation into the larger context of development, specifically into the context of the earlier developmental process of cell determination, when embryonic stem cell lineages are formed and the genetic regulatory programs for cell type-specific gene activation and expression are acquired by stem cells.

The clonal mouse embryo cell line, C3H/10T1/2, clone 8 (10T1/2) provides a unique opportunity to examine the molecular genetic regulation of both the developmental determination of vertebrate stem cell lineages and their subsequent differentiation. 10T1/2 is an apparently multipotential cell line that can be converted by 5-azacytidine into three mesodermal stem cell lineages. These determined proliferative stem cells are stable in culture and retain their ability to differentiate in mitogen-depleted medium. The most significant discovery has been that 10T1/2 lineage determination is under simple genetic control and that the regulatory genes that mediate the formation of myogenic cell lineages, and likely the chondrogenic and adipogenic lineages, can be demonstrated and studied by genomic DNA and cDNA transfection approaches. This paper is a description of the remarkable properties and genetic behaviors of the 10T1/2 cells and a discussion of the insights that future studies of this cell may provide.

Mesodermal Differentiation and Oncogenic Transformation

10T1/2 cells are a clonal cell line established and characterized by Reznikoff et al. (1). They were derived from 14- to 17-day C3H whole mouse embryos, so their tissue of origin is unknown. The cell line was established using the method of Aaronson and Todaro (2), which selects for cells that are sensitive to postconfluence inhibition of cell division. These cells maintain a stable morphology even after long periods in culture. When subconfluent, they appear fibroblastlike with extended cytoplasmic processes. In confluent cultures, the cells form a flat, regular, epithelioidlike sheet. The modal chromosome number is stable at 81, which is hypertetraploid for mice. Expression of C-type retroviruses could not be detected over large pas-

sage numbers, and the cells were not tumorigenic when injected into irradiated and nonirradiated C3H mice. The presence and consistency of these characteristics make this cell line ideally suited for studies of malignant transformation *in vitro* by a variety of chemical and viral agents.

During one such study of malignant transformation by the S-triazine nucleoside analogue of cytidine, 5-azacytidine (5-Aza-CR), which is used as a cancer chemotherapeutic agent, Constantinides et al. (3) observed elongated, multinucleated cells that morphologically resembled myofibers. Further investigation showed that the multinucleated cells expressed biochemical properties of muscle so that they could contract when treated with acetylcholine (3), had receptors to acetylcholine capable of binding α -bungarotoxin, and a calcium-dependent (myosin) ATPase (4). Consistent with these results, other researchers demonstrated the presence of an array of muscle-specific contractile proteins synthesized by 5-Aza-CR-derived myofibers (5,6). Thus, 10T1/2 cells can differentiate and form muscle after treatment with 5-Aza-CR.

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A later report by Taylor and Jones (7) showed that two other mesodermal phenotypes, adipocytes and chondrocytes, were expressed by 10T1/2 cells after treatment with 5-Aza-CR at an optimal concentration identical to that which resulted in the formation of myofibers. There was a temporal order of appearance of the three differentiated phenotypes; myofibers appeared 5 to 6 days after the treated cells had reached confluence, followed by adipocytes about 4 days later, and finally chondrocytes in 9 to 16 days more. In the case of myofibers and chondrocytes, the relative order of appearance is consistent with that observed in developing chicken limb buds (8), suggesting that the developmental timing of differentiation is preserved in this cell line following 5-Aza-CR treatment.

Adipocytes have a morphology typical of multilocular fat cells and can be stained with oil Red-O (7). To confirm the identity of this phenotype, Taylor and Jones (7) measured the accumulation of triglycerides and the activities of two key enzymes of fatty acid synthesis, ATP-citrate lyase and acetyl-CoA carboxylase. Consistent with their evidence, Chapman et al. (9) later showed that an additional enzyme activity typical of mature adipocytes, glycerophosphate dehydrogenase, increased 100-fold and that lipoprotein lipase could be detected immunologically in adipocytes derived from 10T1/2 cells by 5-Aza-CR treatment.

The cartilage cells observed were seen in tightly packed foci of polygonal cells (7). These cells showed metachromatic staining with toluidine blue and incorporation of ³⁵S-sulfate, both of which were reduced with digestion using chondroitinase ABC. These results suggested that the cells synthesized sulfated proteoglycans that are associated with the extracellular matrix of cartilage. In addition, these foci of cells bound antibody against type II collagen (7).

In addition to the mesodermal phenotypes observed, Benedict et al. (10) described low frequency (1.75%) oncogenic transformation of 10T1/2 cells by 5-Aza-CR. The transformed cells had an altered morphology, formed typical type III transformed foci, and formed tumors when injected into immunosuppressed syngeneic mice. The appearance of this additional nonmesodermal phenotype suggests that 5-Aza-CR acts on specific targets, in this case oncogenes, rather than changing the general state of cells, forcing them into a preset mesodermal program. In addition to 5-Aza-CR, 10T1/2 cells can be transformed with a variety of other tumorigenic agents, resulting in cell lines (11,12). Two of these lines were subcloned (7) and treated with 5-Aza-CR and scored for myofiber formation. Both cell lines tested gave rise to multinucleated cells with the same 5-Aza-CR concentration dependency as parental 10T1/2 cells but resulted in a much lower frequency of myofiber formation. Again, these results suggest a specificity of 5-Aza-CR action and demonstrate that the transformed phenotype does not preclude the appearance of at least the myogenic phenotype.

Although adipocytes, myofibers, and chondrocytes are all mesodermal in origin, a multipotential precursor cell has never been isolated in primary culture that gives rise to all three differentiated phenotypes. However, there is

further evidence for a common mesodermal precursor cell using other cloned cell lines that can significantly be induced to express these differentiated phenotypes following treatment with 5-Aza-CR. Taylor and Jones demonstrated that Swiss 3T3 cells, an established fibroblastlike cell line derived from mouse embryos, give rise to all three phenotypes after treatment with 5-Aza-CR in a concentration-dependent manner, but at lower frequencies than 10T1/2 cells (7). In addition, these same researchers showed that an adult mouse prostate cell line, CVP3SC6, also gave rise to adipocytes, myofibers, and chondrocytes when treated with comparable concentrations of 5-Aza-CR, although at significantly reduced frequencies compared to the embryo cell lines (13). Therefore, there is reason to propose that 10T1/2 represents a typical mesodermal multipotential cell and that the results of 5-Aza-CR treatment reflect an authentic mechanism regulating the expression of the mesodermal phenotypes.

Determination into Stable Stem Cell Lineages

The analyses reported thus far adequately demonstrate that 10T1/2 cells can differentiate into specialized cell types following treatment with 5-Aza-CR. However, terminal differentiation is an end point assay and cannot by itself address the question of which developmental decisions are being made as a result of 5-Aza-CR. Are the cells being converted directly from fibroblastlike, apparently undifferentiated, parental cells into muscle, fat, and cartilage cells so that the phenotypic genes are somehow being induced directly by the chemotherapeutic agent? Or, are 10T1/2 cells undergoing a process that more closely resembles the *in vivo* situation so that 5-Aza-CR converts 10T1/2 cells into determined stem cells that can proliferate and then differentiate, expressing their unique cell-type specific proteins?

Konieczny and Emerson (5) undertook a clonal analysis of 5-Aza-CR treated 10T1/2 cells to determine whether the cells were bypassing determination or were in fact proceeding through the entire developmental program. 10T1/2 clonal cultures were treated with 5-Aza-CR, and multiple colonies containing cells of each differentiated phenotype were recloned and expanded.

Proliferative cells from the adipogenic lines morphologically resembled 10T1/2 cells, whereas cells from the myogenic clones were bipolar, small, and highly refractile, and cells from the chondrogenic clones appeared polygonal. The clonal populations were allowed to reach confluence and examined for differentiated cells. In every case, the clonal cell lines isolated differentiated into only the single selected cell type. Two-dimensional protein gel analyses showed that specific proteins present in 10T1/2 cell extracts were missing from the myogenic, adipogenic, and chondrogenic cell lines examined. In addition, at least one major protein appeared to be unique to the myogenic cell lines. Most significantly, muscle-specific contractile proteins could not be detected in extracts from proliferative

cultures of the myogenic cell lines, whereas fusing cultures exhibited the full spectrum of differentiation-specific proteins. These results showed that, following 5-Aza-CR treatment, cells of each phenotype arose that synthesized sets of proteins that were different than those expressed by 10T1/2 cells, the other mesodermal phenotypes, and the end point-differentiated cells. These cells were proliferative, stable, and capable of differentiation under appropriate culture conditions of low mitogen stimulation. Thus, these studies showed that 5-Aza-CR did not directly activate differentiation-specific genes but rather converted the cells into determined stem cell lineages capable of differentiating in the appropriate environment.

Consistent with the clonal and protein analyses of Konieczny and Emerson, Chapman et al. performed a cDNA analysis of an adipocyte clone derived from 10T1/2 cells by 5-Aza-CR (9). These researchers constructed a cDNA library from a fully differentiated adipocyte line and, using differential screening techniques, characterized three classes of RNAs: a) RNAs that are absent in untreated 10T1/2 cells and preadipocytes but are expressed by adipocytes; b) RNAs that are present at low levels in 10T1/2 cells and in preadipocytes and increase 3- to 4-fold or 10- to 20-fold in adipocytes; and c) a unique RNA that is 4-fold more abundant in preadipocytes than in 10T1/2 cells and undergoes an additional 6-fold increase in adipocytes. The expression of several other RNAs decreased during adipogenic conversion. Their results reiterate at the RNA level the individual identities of the 10T1/2 cells, proliferative determined stem cells, and the differentiated phenotypic cells.

Further evidence that 5-Aza-CR is not directly activating muscle-specific contractile protein genes was provided by DNA transfection experiments. 10T1/2 cells and a previously characterized myogenic cell line derived from 10T1/2 cells were stably transfected with a quail skeletal troponin I (TnI) gene (14). Neither the quail TnI gene nor the endogenous mouse skeletal α -actin gene was expressed in parental 10T1/2 cells, a myoblast line derived from the transfected 10T1/2 cells by 5-Aza-CR treatment, or the proliferative myogenic cell line. In contrast, these genes were expressed in fusing cultures of the myogenically converted, transfected 10T1/2 cells and the myogenic cell line. Similar results were obtained when 10T1/2 cells and a 5-Aza-CR-derived myogenic cell line were stably transfected with a chicken cardiac α -actin gene (6). These results proved that 5-Aza-CR is not directly affecting contractile protein genes; the exogenously added genes were not expressed in proliferative cells even when they were introduced prior to 5-Aza-CR, but the genes were expressed by fusing cells even when they were introduced long after the cell lines had been established by 5-Aza-CR treatment. The transfected genes were activated appropriately, both temporally and quantitatively, in fusing myofibers, suggesting that they are responsive to the regulatory mechanisms operating in cells converted by treatment with 5-Aza-CR. Therefore, these experiments suggest that 5-Aza-CR is involved in establishing the regulatory program that controls the expression of the differentiation-specific genes.

It is evident from the work reported that 5-Aza-CR treatment of 10T1/2 cells can result in the establishment of distinct cell lines that fulfill the criteria of being stably determined, proliferative stem cell lines capable of differentiating and expressing their specific phenotypic genes. Further, the evidence indicates that 5-Aza-CR is not activating the differentiation-specific genes themselves but rather is inducing a determined state that activates a regulatory program capable of responding appropriately to environmental signals. In short, it appears that 5-Aza-CR is causing cell determination of certain lineages within 10T1/2 cells so that they at some point must represent multipotential, mesodermal precursor cells. It is not possible at this point to determine whether 10T1/2 cells are actually multipotential or whether 5-Aza-CR induces an unstable multipotential state.

Conversion of DNA Sequences by Hypomethylation

Several lines of evidence suggest that 5-Aza-CR converts 10T1/2 cells into determined lineages by causing hypomethylation of DNA. Jones and Taylor (15) showed by direct measurements of DNA methylation that 5-Aza-CR at micromolar concentrations is an efficient inhibitor of methylation in 10T1/2 cells. Decreases in methylation could be correlated with increasing 5-Aza-CR doses and with increasing myofiber formation. In these experiments the optimal concentration of 5-Aza-CR for myofiber formation was 2 μ M, which caused a 62% decrease in cytosine methylation. Additional direct measurements showed that newly synthesized strands were significantly undermethylated when the cells were exposed to 5-Aza-CR for 20 hr and were not remethylated during a subsequent 20-hr period in the absence of 5-Aza-CR.

Consistent with the hypothesis that 5-Aza-CR acts by causing hypomethylation of DNA, the deoxy analogue, 5-aza-2'-deoxycytidine, causes myofiber formation at the same frequency but with 1/10 the concentration. Other analogues of cytidine substituted at the same position were shown to be effective inhibitors of DNA methylation and also to cause myofiber formation. Although the level of incorporation of 5-Aza-CR reached only 5% when there was an 80 to 85% inhibition of methylation, it was hypothesized that the methylating enzymes walk along the DNA so that 5-Aza-CR may impede enzyme movement and therefore inhibit methylation of additional sites.

Maximal induction of myofibers and adipocytes was shown to occur when 10T1/2 cells were treated with 5-Aza-CR during early S phase (4,13). Incorporation of the analogue into DNA was not enhanced during this period of the cell cycle and there were no significant differences in incorporation into RNA throughout the cell cycle. Therefore, it was theorized that incorporation of 5-Aza-CR into specific DNA sequences synthesized during early S-phase is responsible for the conversion events.

Studies of methylation patterns by Bird (16) showed that both cytosines in a CpG paired sequence are either both methylated or both unmethylated, which is consistent with theories that methylation patterns are conser-

vatively replicated (17,18). A model proposed by Jones and Taylor (13) suggests that 5-Aza-CR would perturb the methylation patterns in 10T1/2 DNA and that these new patterns of methylation would become set within the genome during two DNA replication cycles. This model would predict the subsequent reports that clonal cultures of 5-Aza-CR treated 10T1/2 cells contain mixed colonies of converted and parental cells (5) or more than one converted phenotype (5,13) and would predict the subsequent segregation and stability of phenotypes (5) following recloning. Although there has been no direct demonstration that hypomethylation of specific DNA loci by 5-Aza-CR results in conversion of 10T1/2 cells, these results strongly support this mechanism of action.

The high frequency of conversion makes the 10T1/2 cell line a particularly attractive model system in which to study determination. In addition, these frequencies suggest a model that proposes a simple genetic basis for determination. Taylor and Jones (13) treated single cells with 5-Aza-CR and examined the expanded cultures for phenotypic conversion. A total of 62% of the colonies showed phenotypic conversion with at least one differentiated cell type (Table 1). Of these colonies, 31% contained myofibers, 8% contained adipocytes, and 2% contained chondrocytes. In addition, all combinations of differentiated phenotypes were found in mixed colonies. Surprisingly, mixed muscle and adipocyte colonies comprised 16% of the total, whereas the other mixed phenotypes were 1 to 2% of the total. Konieczny et al. (5,19) treated cultures of 10T1/2 cells plated at clonal density with 5-Aza-CR (Table 1). They found that a total of 34% of the colonies contained differentiated cells; 25% of the clones contained myofibers, 7% had adipocytes, and 1% contained chondrocytes. An additional 1% of the colonies contained both muscle and fat. In each case, the colonies containing differentiated cells also contained parental 10T1/2, as did the remaining 66% of the colonies. When the colonies with converted cells were recloned, the individual phenotypes segregated. These separate reports show that there is a high frequency of mesodermal determination, especially myogenic determination. The discrepancies in the reported frequencies of mixed colonies have not been resolved.

Konieczny et al. proposed a model to account for these high frequencies of determination (5). They suggested

that 10T1/2 cells were blocked in mesodermal conversion by DNA methylation of unlinked determination loci. These loci could have been methylated in the embryonic cell from which the 10T1/2 cell line was derived or during the establishment of the line in culture. During early S-phase, some of the determination loci would be hemimethylated when the cells were treated with 5-Aza-CR. During the subsequent two rounds of replication and random segregation of chromosomes, the daughter cells would receive either fully unmethylated sites at these loci or methylated sites identical to parental 10T1/2 DNA. If the number of unmethylated sites were adequate, a determination locus would be activated and a portion of the daughter cells would be converted to the corresponding new phenotype. Simply correlating the level of hypomethylation to conversion frequencies suggests that, in the case of myogenic conversion, there are only one or a few determination loci. That is, if DNA methylation were 60% inhibited in a random manner at the concentration of 5-Aza-CR used, 36% of the colonies would contain myogenic cells, assuming that two unmethylated sites within the myogenic locus or one site each within two loci were required for conversion. The lower frequencies of adipogenic and chondrogenic conversions suggest that they require correspondingly more unmethylated sites within a locus or multiple loci. This model is consistent with the current theories of DNA methylation (17,18) and explains the segregation and stability of phenotypes from the clonal analysis of 5-Aza-CR treated 10T1/2 cells.

Myogenic Determination

The establishment of stable clonal cell lines of each mesodermal lineage following 5-Aza-CR treatment of 10T1/2 cells suggested a preliminary test of the theory that determination has a simple genetic basis such that conversion is caused by the activation of one or a few regulatory loci (5). Transfected genomic DNA from a cell with an active, presumably hypomethylated, determination locus should convert 10T1/2 cells to a mesodermal lineage. Konieczny et al. (19) isolated genomic DNA from a 10T1/2-derived myoblast cell line and from embryonic quail myoblasts. Each genomic DNA was cotransfected with a plasmid containing a gene conferring resistance to the antibiotic G418. In each case, the frequency of myogenic conversion was 2×10^{-4} G418-resistant cells. In addition, Lassar et al. (6) demonstrated in a similar transfection experiment that transfection of 10T1/2 with genomic DNAs isolated from a 10T1/2-derived myoblast cell line or from the mouse C2C12 myoblast cell line resulted in myogenic conversion frequencies of about 7×10^{-5} G418-resistant cells. These experiments, although suggestive, are limited in their interpretations. Since no marker was associated with the transfected genomic DNA, there was no method to confirm that the exogenously added DNAs were responsible for the observed conversion events. In fact, Lassar et al. reported a high frequency of spontaneous conversion (6).

A more direct test of the model has been performed by

Table 1. Phenotypic conversion frequencies of 10T1/2 cells following 5-azacytidine treatment.

Phenotype observed	Frequency, % ^a	Frequency, % ^b
Muscle	31	25
Adipocyte	8	7
Chondrocyte	2	1
Muscle and adipocyte	16	1
Muscle and chondrocyte	1	0
Adipocyte and chondrocyte	2	0
Muscle, adipocyte, and chondrocyte	2	0

^aTaken from work by Taylor and Jones (13). Single cell cultures were treated with 3 μ M 5-Aza-CR and a total of 173 surviving colonies were examined.

^bTaken from work by Konieczny and Emerson (5). Clonal cultures were treated with 3 μ M 5-Aza-CR and a total of approximately 1500 colonies were examined.

Pinney et al. (20). A human DNA library cloned into a cosmid vector was transfected into 10T1/2 cells and selected for G418 resistance conferred by a neomycin resistance (*Neo^R*) gene contained within the vector. The rationale for this experiment was that cloned human DNA passaged through methylase-deficient bacteria would be hypomethylated and therefore mesodermal determination genes may be active when introduced into mammalian cells. In addition, the *Neo^R* gene would allow tracking of linked DNA in primary and secondary transfections. The frequency of myogenic conversion in primary transfections was 2×10^{-4} G418 resistant colonies. Considering the average complexity of cosmid integrations, this frequency showed that a single gene was sufficient to cause myogenic conversion. One of the myogenic clones was isolated as a stable myoblast cell line and genomic DNA isolated for secondary transfections. 10T1/2 cells were transfected with this genomic DNA and selected for the *Neo^R* gene that should remain linked to the myogenic determination gene. In two transfection experiments, two stable myogenic cell lines were obtained out of a total of 31 G418 resistant colonies. These results prove that a single DNA locus, *myd* is sufficient to cause myogenic determination and, therefore, that there is a simple genetic basis for myogenic conversion.

Using an alternative cDNA approach, Davis et al. have identified and characterized a gene product that causes myogenic transformation of 10T1/2 cells when the cDNA is cloned into an expression vector (21). A cDNA library was constructed from a mixture of myoblast and myofiber poly A⁺ RNA from a 10T1/2-derived myogenic cell line. A differential screening approach was used to identify three clones that were expressed primarily in proliferating myoblasts but were absent in myogenic variants that had reverted to a nonfusing phenotype. One of these clones, Myo D, is not expressed in confluent, growth-arrested 10T1/2 cells and is absent or greatly reduced in the nonfusing variant lines.

In contrast, Myo D is expressed by proliferative and differentiated 10T1/2-derived myogenic cell lines. Interestingly, Myo D RNA is expressed in neonatal and adult mouse skeletal muscle but in none of the other tissues tested. When cloned into an expression vector under the control of the Moloney sarcoma virus LTR, Myo D converts 50% of transfected 10T1/2 cell colonies as well as several other mouse fibroblast cell lines [NIH 3T3, Swiss 3T3, Swiss 3T3 clone 2 (C2), L Cells, Swiss 3T3-derived adipocyte lines (3T3-LI and 3T3-F442A), and a 10T1/2-derived adipocyte line (TA1)] at frequencies varying from 3 to 65%. It is interesting and perhaps significant that the adipogenic phenotype, just as the transformed phenotype (7), did not preclude myogenic conversion. No myogenic conversion was observed when a monkey kidney cell line (CV1) was transfected. The authors noted that the lack of conversion of CV1 cells could be due to the epithelial, rather than mesodermal, origin of these cells or a species incompatibility of the Myo D product. Thus, a single gene product can cause conversion of 10T1/2 cells as well as other embryonic cell lines of apparently mesodermal origin even those considered

to be already determined.

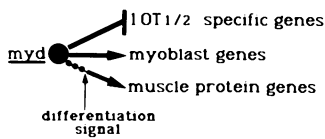
These two experimental approaches have yielded intriguing results and may indicate that a single gene encoding a single gene product is necessary and sufficient to convert 10T1/2 cells into a myogenic lineage. Alternatively, if *myd* and the Myo D genes are not identical, these separate results may suggest a pathway of genes involved in myogenic conversion. The genomic DNA approach suggests that the *myd* locus contains the promoter and any regulatory elements necessary for expression within the 10T1/2 cells and that after passage through the bacterial host the *myd* locus is in an active state. Further experimental data are required to conclude that *myd* is activated by hypomethylation or that it is the initiator or sole regulator of myogenic conversion. Similarly, it can be inferred that the Myo D product is an integral part of the myogenic conversion process, yet because its expression is under the control of a heterologous, constitutive promoter, it is unclear whether the Myo D gene is the cause or rather is the result of the myogenic conversion event. Ongoing research should be able to address and answer most of these questions.

Proposed Models of Myogenic Determination

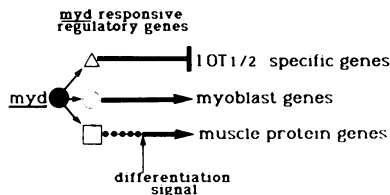
The studies reviewed in this article provide compelling evidence that conversion of 10T1/2 cells into determined stem cell lineages has a simple genetic basis. In the case of myogenic conversion, determination appears to be controlled by activation of a single determination locus, which for the sake of discussion will be called *myd*, probably by a hypomethylation mechanism. The activation of this myogenic determination gene causes profound changes in gene expression, suggesting that the *myd* locus is responsible for establishing a new regulatory program that must include repression of the synthesis of a specific set of 10T1/2 cell proteins, activation of the synthesis of myoblast-specific proteins, and preparation of a transcriptional regulatory program for the differentiation-specific activation of muscle protein genes.

We can imagine three general mechanisms by which a myogenic determination gene might establish the regulatory changes involved in the myoblast determination process. The first is a master regulatory gene mechanism in which the product of *myd* gene acts directly upon all three classes of determination-specific genes, i.e., to repress 10T1/2 cell-specific genes, to induce myoblast genes, and to prepare a transcriptional control system for the activation of muscle genes (Fig. 1A). The second is a parallel regulatory gene pathway mechanism in which *myd* initiates, and perhaps maintains, a secondary set of regulatory genes that would, in turn, control the myoblast-specific processes (Fig. 1B). A third possibility is a hierarchical pathway model in which *myd* would induce secondary regulatory genes, which would subsequently activate a tertiary set of muscle-specific regulatory genes (Fig. 1C). In these cases, the function of such activators of muscle protein genes could be regulated by

A. MASTER REGULATORY GENE MODEL



B. PARALLEL PATHWAY MODEL



C. HIERARCHICAL PATHWAY MODEL



FIGURE 1. Models of myogenic determination.

environmental cues related to concentrations of growth factors (22,23). Such growth factor regulation could be accomplished by a number of mechanisms such as control of the synthesis and the levels of muscle protein gene activators, the formation of transcription complexes with differentiation-specific regulatory proteins, or the post-translational modification of these muscle protein gene transcription activators. Investigation of whether myogenic determination gene expression is constantly or transiently required to maintain the myogenic lineage could help distinguish among these mechanisms. In this regard, Davis et al. showed that Myo D is expressed continually in myogenically converted cell lines and that lines that revert to a nonmyogenic phenotype can be rescued by transfection with Myo D cDNA in an expression vector (21). Since myogenic determination seems primarily to involve transcriptional control processes, it may be that the *myd* product is a transcription factor. However, it is conceivable that *myd* could regulate transcription less directly as a cell surface receptor or as an intermediate in a signal transduction pathway. Therefore, discovering the nature of the *myd* product will provide valuable insight into the exact mechanism involved in establishing myogenic lineages.

Significant progress has been made in understanding the molecular mechanisms involved in cell lineage determination and differentiation using the 10T1/2 cell line. The remarkable properties of 10T1/2 cells suggested models of determination and the advantages of a clonal tissue culture system have allowed researchers to test these hy-

potheses using DNA transfection approaches. Perhaps the most outstanding conclusion reached is that a developmental decision, myogenic determination, is under simple genetic control and may primarily involve the establishment of transcriptional regulatory programs. It is reasonable to expect that similar mechanisms will be discovered that regulate the establishment of adipogenic and chondrogenic lineages in the 10T1/2 cell and that the approaches used to investigate myogenic determination can be applied. Furthermore, the experimental approaches and findings developed in studies of 10T1/2 cells offer a new paradigm for both *in vitro* and *in vivo* investigations of embryonic determination in a variety of different embryonic lineages.

We thank Brian Brunk and Patrick Bender for helpful discussions.

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